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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/53, A61K 38/00 — A1

(11) International Publication Number: WO 00/03245

(43) International Publication Date: 20 January 2000 (20.01.00)

(21) International Application Number: PCT/US99/11844

(22) International Filing Date: 28 May 1999 (28.05.99)

(30) Priority Data:
60/087,107 28 May 1998 (28.05.98) US

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(81) Designated States: AU, CA, JP, KR, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: PEPTIDE LIGANDS FOR THE HUMAN FIBROBLAST GROWTH FACTOR (FGF) RECEPTOR

(57) Abstract

Clones isolated from phage display libraries that bound to a human Fibroblast Growth Factor (FGF) receptor probe are disclosed. Polypeptides unrelated to the primary structure of FGF and encoded by DNA sequences of the phage clones that bound to the FGF receptor are disclosed. Phage clones derived from one FGF receptor-binding clone were isolated and sequenced, and a 13-mer consensus amino acid sequence common to these polypeptides, but unrelated to the primary structure of FGF, are disclosed.

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PEPTIDE LIGANDS FOR THE HUMAN FIBROBLAST GROWTH FACTOR (FGF) RECEPTOR

Field of the Invention

The present invention relates to the fields of pharmacology and drug discovery. More particularly, the invention relates to novel peptide compositions that can bind to the human fibroblast growth factor receptor (FGFR), and to methods of making small molecule non-peptide agonists or antagonists of the FGFR using such peptide compositions as design templates.

Background of the Invention

Drug discovery traditionally has relied upon high-throughput screening of large numbers of chemical compounds to identify novel drug leads. More recently, combinatorial libraries constructed by chemical or biological means have greatly expanded the number of compounds available for screening. Biological libraries, such as phage displayed peptide libraries, of random directed semi-random sequences represent particularly rich sources of molecular diversity and advantageously possess the ability to self-replicate. With a self-replicating system, the search for high affinity leads is not limited to members that happen to be present in the initial library. As discussed more fully below, desired characteristics of initial sequences can be greatly improved by employing successive rounds of mutagenesis, affinity selection, and amplification. These approaches recently have been used to discover small peptides capable of binding cytokine receptors.

The binding of cytokines to their receptors plays a fundamental role in haemostasis, immune regulation and basic cellular processes essential for multicellular life. Human Fibroblast Growth Factor (huFGF) is a cytokine that stimulates the growth of human fibroblasts. A variety of forms of human FGF are known including acidic FGF (aFGF), basic FGF (bFGF), human FGF-4, human FGF-5 and human FGF-6, many of which have been produced recombinantly and are commercially available(Gospodarowicz, D. *et al.*, 1974, *Proc. Natl. Acad. Sci. USA* 71:4648; Gospodarowicz, D. *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81:6963; Esch, F. *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:6507; Gimenez-Gallego, G. *et al.*, 1985, *Science* 230:1385; Sigma Chemical

Co. 1996 Catalog, pp. 1591, 1677). When used therapeutically, such as to stimulate wound healing or promote angiogenic capability, huFGF may be administered intravenously or by subcutaneous injection. The fact that huFGF is a relatively large protein adversely impacts the cost of manufacture, the pharmacological properties of molecule, and the mode of delivery of this therapeutic agent.

The human Fibroblast Growth Factor Receptor (FGFR) belongs to the cytokine receptor superfamily whose members share common structural features including an extracellular ligand binding domain, a single transmembrane-spanning region, and an intracellular cytoplasmic domain. The extracellular domain (ECD) is sufficient to mediate receptor-ligand binding. It is therefore possible through recombinant DNA techniques to synthesize DNA encoding the ECD as a fusion with secreted proteins to produce reagents useful for identifying receptor binding molecules, for example by screening a phage display library.

Phage display libraries expressing fusions of random or semi-random peptides and bacteriophage coat proteins represent convenient versions of combinatorial libraries that can be screened to identify receptor ligands. Upon infection and assembly of phage particles, the random polypeptides are outwardly disposed for interaction with antibodies or other receptor probes. The display of peptides on filamentous bacteriophage has provided a general method for selecting amino acid sequences that have desirable binding characteristics from the large repertoires of phage libraries. Large libraries of random peptides having a diversity of about 10^8 can be screened using affinity selection methods to identify useful peptide ligands. In such libraries, the random peptide or protein sequences are generally displayed as fusion proteins, such as with the pIII phage coat protein of bacteriophage M13.

Because the phage particles that are selected contain the nucleic acid that encodes the fusion protein, the genetic information which identifies the amino acid sequence of the fusion protein is physically linked to the protein, making sequence determination relatively easy. General methods of constructing and screening phage display libraries have been described previously (*e.g., see* Sawyer *et al.*, *Protein Engineering* 4:947-953 (1991);

Akamatsu *et al.*, *J. Immunol.* 151:4651-59 (1993), Smith et al., *Methods in Enzymol.* 217:228-257 (1993); Clackson et al., *Trends Biotechnol.* 12:173-184 (1994), and U.S. Patent No. 5,427,908 to Dower *et al.*).

Phage display library screening has been used to isolate polypeptides that inhibit the binding of FGF to the FGFR (Yayon et al., *Proc. Natl. Acad. Sci. USA*, 1993, 90(22):10643-10647. The sequences of the isolated polypeptides are not similar to those described herein. Several peptides that are truncated forms of human native FGF are commerically available (Bachem Co., 1998 Catalog, pp. 539-540). These peptides are unlike the amino acid sequences of the peptides disclosed herein.

Despite progress in identifying FGFR ligands, there remains a need to identify ligands that exhibit receptor-binding properties but do not resemble the native huFGF primary structure. Such peptides may serve as molecular models for non-peptide small molecule agonists or antagonists of the human FGFR.

Summary of the Invention

According to one aspect of the invention, there is disclosed an isolated polypeptide capable of binding to a human Fibroblast Growth Factor receptor, having the formula $CX_1X_2LX_3X_4GAPFX_5X_6X_7X_8C$ wherein X_1 is S or R, X_2 is any natural α-amino acid, X_3 is a neutral and hydrophobic α-amino acid, X_4 is any natural α-amino acid, X_5 is H or Q, X_6 is a branched-chain amino acid, X_7 is a neutral and hydrophobic or a basic α-amino acid, and X_8 is an acidic amino acid or a neutral and polar amino acid. In preferred embodiments of the isolated polypeptide, X_2 is a neutral and hydrophobic, neutral and polar, or acidic amino acid, and more preferably is A, V, G, P or D. In preferred embodiments, X_3 is a branched-chain amino acid or F, and more preferably is L, I or F. In preferred embodiments, X_4 is a branched-chain amino acid, E, R or A, and more preferably is V or E. In preferred embodiments, X_6 is V or L, and more preferably is V. In preferred embodiments, X_7 is P, A or R, more preferably is P or A, and most preferably is P. In preferred embodiments, X_8 is E, D or G, more preferably is E or D, and most preferably is E. In other embodiments, the isolated polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:37,

SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47 and SEQ ID NO:49, and more preferably has the amino acid sequence of SEQ ID NO:41.

Also included in the invention is a method of activating a human Fibroblast Growth Factor (FGF) receptor, comprising the steps of contacting a cell having a human FGF receptor on it surface with at least one purified polypeptide as described above and allowing the polypeptide to bind to the human FGF receptor, thereby initiating activation of the human FGF receptor. Preferably, the polypeptide used in this method has the amino acid sequence of SEQ ID NO:23, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, or is a combination of these polypeptides. Another method of this invention is a method of activating a human Fibroblast Growth Factor (FGF) receptor, comprising the steps of providing a polypeptide as described above, synthesizing and purifying a peptide memetic modeled on the polypeptide, contacting a cell having a human FGF receptor on it surface with the purified peptide mimetic, and allowing the peptide mimetic to bind to the human FGF receptor, thereby initiating activation of the human FGF receptor. Another embodiment is a method of inhibiting binding of a human Fibroblast Growth Factor (FGF) to a human Fibroblast Growth Factor receptor, comprising the steps of providing a polypeptide as described above, in sufficient quantity to compete with a human FGF for binding to a human FGF receptor, and allowing the polypeptide to bind to the human FGF receptor, thereby inhibiting binding of a human FGF to the FGF receptor. A preferred embodiment of this method uses the polypeptide having the amino acid sequence of SEQ ID NO:41. One embodiment is a method of inhibiting binding of a human Fibroblast Growth Factor (FGF) to a human FGF receptor, comprising the steps of providing a polypeptide as described above, synthesizing and purifying a peptide memetic modeled on the polypeptide, contacting a cell having a human FGF receptor on it surface with the purified peptide mimetic, and allowing the peptide mimetic to bind to the human FGF receptor, thereby inhibiting binding of a human FGF to the human FGF receptor on the cell.

Another aspect of the invention is a method of discovering drugs that mimic human Fibroblast Growth Factor (FGF). This method includes the steps of constructing a phage display library in which a fusion protein comprising a polypeptide consisting of a random sequence of amino acids and a phage protein; screening the phage display library for at least one clone that binds to a human FGF receptor probe, isolating an initial clone that binds to the human FGF receptor probe, determining a nucleic acid sequence of the initial clone that codes for the polypeptide contained within the fusion protein, constructing an evolved phage display library by mutagenesis *in vitro* of the nucleic acid sequence of the initial clone, isolating additional clones that bind to a human FGF receptor probe from the evolved phage display library, determining nucleic acid sequences from the additional clones isolated from the evolved phage library for individual sequences that code for a binding polypeptide contained within each additional clone 's fusion protein, thereby determining an amino acid sequence of each binding polypeptide, comparing at least two amino acid sequences of two different binding polypeptides to identify a consensus amino acid sequence capable of binding to a human Fibroblast Growth Factor receptor, and synthesizing a compound that includes or mimics the consensus amino acid sequence. In one embodiment of this method, the human FGF receptor probe is a chimeric protein comprising a human FGF receptor amino acid sequence covalently linked to a mammalian immunoglobulin heavy chain amino acid sequence. In another embodiment, the synthesizing step synthesizes an organic compound, which is preferably a polypeptide having an amino acid sequence unlike a contiguous sequence of amino acids in human FGF. More preferably, the polypeptide is a cyclic polypeptide.

Another aspect of the invention is an isolated DNA sequence coding for a polypeptide capable of binding to a human FGF receptor, selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46 and SEQ ID NO:48.

One more aspect of the invention is an isolated polypeptide capable of binding to a human FGF receptor containing an amino acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47 and SEQ ID NO:49. Preferably, the isolated polypeptide is a cyclic polypeptide.

Brief Description of the Drawings

FIG. 1A is a line graph showing the relative affinities of isolated phage clones for a human FGFR-IgG fusion protein as measured by an ELISA (O.D.) at different phage dilutions containing equivalent plaque forming units (pfu) for phage clones displaying: 43-mer peptides (clone 83, ×; clone 73, ●), 38-mer peptides (clone 64, ◆; clone 66, ▲) and 13-mer peptides (clone 13-1, ■; clone 13-9, *; clone 13-10, +; and clone 13-12, ▬).

FIG. 1B is a line graph showing the relative affinities of isolated phage clones for a human FGFR-IgG fusion protein as measured by an ELISA (O.D.) at different phage dilutions containing equivalent plaque forming units (pfu) for phage clones displaying: 43-mer peptides (clone 94, ▲; clone 74, ×), 38-mer peptides (clone 64, ◆; clone 61, ▬) and 13-mer peptides (clone 13-1, ■; clone 13-3, *; clone 13-6, ●; and clone 13-5, +).

FIG. 2 schematically shows the mutagenesis approach that was used to create an evolved peptide library from the FGFR-binding clone 13-1. The upper line shows the amino acid sequence of the clone 13-1 peptide (SEQ ID NO:23) that was part of the pIII fusion protein. The nucleic acid sequence thereunder shows the upper strand of a series of oligonucleotides that were synthesized in which the bases conserved in all the clones are shown in capital letters, and the bases shown in small letters represent the redundant sequences where "g" represents residues synthesized with a mixture of 73% G and 9% of each of A, T and C; "a" represents residues synthesized with 73% A and 9% of each of G, T and C; "t" represents residues synthesized with 73% T and 9% of each of G, A and C; "c" represents residues synthesized with 73% C and 9% of

each of G, A and T; and "s" represents residues synthesized with 50% C and 50% C.

FIG. 3 is a line graph showing the relative affinities of several independent phage clones for the FGFR-IgG fusion protein relative to that of clone 13-1, as measured by ELISA ("OD") using equivalent numbers of pfu for each clone ("PFU Dilution").

FIG. 4 is a line graph showing results of a competitive inhibition ELISA assay to measure inhibition of FGFR binding to FGF-coated plates, where the percentage of inhibition ("Percent Inhibition") was determined for different concentrations of inhibitor ("Inhibitor Dilution"), using as inhibitors the clone 17 peptide (-▲-), a negative control peptide (--▲--) that does not bind to FGFR, and, as the positive control, recombinant human basic FGF protein (■).

Detailed Description of the Invention

Herein we disclose novel peptides that can bind and activate the human FGFR and methods of identifying and isolating such peptide ligands that may serve as design templates for making small molecule analogs of huFGF. Unexpectedly, the primary amino acid sequences of these peptide ligands was unrelated to the primary amino acid sequence of the native human FGF protein. Moreover, the peptides disclosed herein also are unrelated to previously isolated FGFR binding peptides from phage display libraries. The human FGFR-binding peptides described herein can be candidate therapeutic agents which act as receptor agonists or antagonists for treating humans and animals, and may also be used as models for designing small molecules having agonist or antagonist activities. Short peptides or small molecule peptide mimetics that stimulate human fibroblast growth would have advantages over native or recombinant human or animal FGF with respect to convenience of therapeutic delivery, pharmacokinetics and stability, and production costs. Such peptides and peptide mimetics may also be convenient and cost-effective synthetic alternatives to recombinant FGF or FGF isolated from animal tissue for use as growth factors in *in vitro* tissue culture. Such peptides and non-peptide small molecules that mimic the biological properties of human FGF are desirable additions to the pharmacopeia.

A collection of peptides that bind the human FGFR and mimic the pharmacological activity of human Fibroblast Growth Factor were identified by screening four different phage display libraries, each displaying different lengths of random amino acids fused to the N-terminus of the pIII capsid protein of M13. The screening assay used a chimeric protein containing an amino acid sequence of the human FGFR covalently linked to an IgG sequence (referred to as a "human FGFR-IgG fusion protein") to select phage bearing a pIII fusion protein capable of binding to the FGFR. One FGFR-binding phage display clone that was initially isolated from a phage library displaying 13 random amino acids was used to create an "evolved library" in which cyclic pIII fusion proteins were generated containing amino acid substitutions compared to the amino acid sequence of the initially-isolated clone. The evolved library contained a variety of amino acid substitutions over the entire length of the peptide which were screened using biopanning to isolate additional FGFR-binding clones. The sequences coding for the binding peptides, and non-binding controls, were determined. Neither the initial isolate nor any of the FGFR-binding clones isolated from the evolved library shared significant sequence similarity with the primary amino acid sequence of human FGF. The sequences of the FGFR-binding clones isolated from the evolved library included a consensus amino acid sequence of $CX_1X_2LX_3X_4GAPFX_5X_6X_7X_8C$, (SEQ ID NO:1) that codes for a 15-mer cyclic peptide in which X_1 is S or R, X_2 is any natural α-amino acid, X_3 is a neutral and hydrophobic α-amino acid, X_4 is any natural α-amino acid, X_5 is H or Q, X_6 is a branched-chain amino acid, X_7 is a neutral and hydrophobic or a basic α-amino acid, and X_8 is an acidic amino acid or a neutral and polar amino acid. The consensus sequence includes a 13-mer sequence flanked by two terminal cysteine residues used to produce the cyclic peptide by disulfide bridging, and four central invariant residues, although the other amino acid residues probably also contribute to binding of FGFR.

An exemplary synthetic peptide, derived from a purified phage clone isolated from the evolved phage library, inhibited binding of the chimeric FGFR-IgG to an immobilized FGF in an *in vitro* assay.

Briefly, the human FGFR ligands disclosed herein were identified by a multi-step procedure that involved: (1) creating a chimeric receptor probe that

binds human FGF; (2) creating phage display libraries expressing a large number of target random peptides varying from 9 to 43 amino acids; (3) screening the libraries with the chimeric human FGF receptor probe to identify one or more lead peptides having FGF receptor-binding activity; and (4) creating and testing derivatives of a lead peptide in an evolved library to identify structural features common to additional peptides of similar size that bind to human FGF receptor probe. Certain general features of the methods that were used to obtain the novel peptides that bind to human FGFR are described below, with detailed descriptions of the methods and results presented in the Examples.

Definitions

By "chimeric protein" is meant a non-naturally occurring protein or polypeptide comprising some or all of the amino acid sequences from at least two different proteins or polypeptides, or of one protein or polypeptide and a non-naturally occurring polypeptide chain. As used herein, a chimeric protein is designed, constructed by genetic engineering, synthesized, or otherwise selected intentionally, and contains at least two domains, each having some structural and/or functional characteristic that is not present in the other domain.

By "directly or indirectly labeled" is meant that a molecule may contain a label moiety which emits a signal which is capable of being detected directly (e.g., radioisotope, dye, or fluorescent or chemiluminescent moiety), or may contain a moiety which, through some additional reaction (i.e., indirectly), is capable of being detected (e.g., an attached enzyme, ligand such as biotin, enzyme substrate, epitope, or nucleotide sequence).

By "secondAry molecule" is meant a molecule which is able to bind to at least a portion of the second domain of a chimeric protein, thereby allowing detection or purification of the chimeric protein.

By "hinge region" is meant one of a family of proline and cysteine containing amino acid sequences that occur between the C_{H2} and C_{H1} regions of many mammalian immunoglobulin (Ig) heavy chains, or analogs of these amino acid sequences, in which the regions to the amino- and carboxyl-terminal sides of the hinge are spatially separated by a turn or kink in the polypeptide chain to facilitate separate and simultaneous specific binding to other molecules.

By "ligand" is meant a molecular structure or multimeric molecular complex which can specifically bind to another molecular structure or molecular complex (*i.e.*, its target). Often, though not necessarily, a ligand is soluble while its target is immobilized, such as by an anchor domain in a cell membrane.

By "receptor" is meant at least a portion of a molecular structure or multimeric molecular complex which, in its native environment, has an anchor domain embedded into a cell membrane and is able to bind another molecular structure or complex (*i.e.*, its ligand). Often a receptor is capable of transducing an intracellular signal in response to ligand binding. Many receptors have higher affinity for a ligand when either or both the receptor or its ligand are in a homomultimeric or heteromultimeric form (*e.g.*, a dimer).

By "solid support" is meant an insoluble matrix that is either biological in nature (*e.g.*, a cell, bacteriophage or biological polymer such as cellulose) or synthetic (e.g., a synthetic polymer, such as an acrylamide derivative, nylon, silica, and magnetic particles) to which soluble molecules may be linked or joined.

By "modified" is meant non-naturally occurring or altered in a way that deviates from naturally-occurring compounds.

By "molecular evolution" is meant a process of creating a library of variant peptides by randomization, at a controlled rate, of a nucleic acid sequence coding for a lead peptide having desired functional characteristics.

By "molecule" is meant a molecular-sized inorganic or organic compound, such as, for example, a peptide, protein, nucleic acid, fat or fatty acid, which may be naturally ocurring or synthetically produced.

By "multimeric molecular complex" is meant a complex comprising at least two molecular components, which individually may be, for example, a peptide, protein, nucleic acid, fat or fatty acid, which are held together by covalent bonds, non-covalent bonds or other known chemical interactions.

Amino acids, described by either their three letter (or one letter) abbreviations, are classified according to the nature of their side-groups (as described in *Genes V*, B. Lewin (Oxford University Press, Inc., New York, NY, 1994). These groups are: "neutral and hydrophobic" which includes Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Trp (W), Phe (F) and Met (M); "neutral and polar"

which includes Gly (G), Ser (S), Thr (T), Tyr (Y), Cys (C), Glu (Q) and Asn (N); "basic" which includes Lys (K), Arg (R) and His (H); and "acidic" which includes Asp (D) and Glu (E). "Branched-chain amino acids" refers to I, L and V.

Unless defined otherwise, all scientific and technical terms used herein have the same meaning as commonly understood by those skilled in the relevant art. General definitions of many of the terms used herein are provided in Dictionary of Microbiology and Molecular Biology, 2nd ed. (Singleton et al., 1994, John Wiley & Sons, New York, NY), and The Harper Collins Dictionary of Biology (Hale & Marham, 1991, Harper Perennial, New York, NY), and in protocol manuals cited herein.

A Chimeric Receptor Probe Having FGF-Binding Activity

Previously used methods for identifying receptor ligands have relied on the binding of anti-ligand antibodies or subfragments of native ligands which may inherently bias the outcome of the screening assay. For example, because linear peptides of only 6 to 10 amino acids can be accommodated in an antibody combining site, it is difficult to identify peptides that include tertiary structural features of receptor-binding ligands. Similarly, subfragments of known ligands often do not include or represent critical higher-order structural features of the cognate ligand. For example, tertiary structure of receptor-binding ligands might not be completely contained in contiguous amino acids of a ligand subfragment.

To avoid structural limitations of combinatorial molecules that may be identified in a binding assay, we created a probe that contained the extracellular domain the native human FGF receptor. For convenience in the screening assay, we created a chimeric probe capable of binding a human FGF receptor ligand, and capable of binding a secondary probe. The chimeric protein incorporated a ligand binding domain of the human FGF receptor ("FGFR") and portions of the murine IgG1 antibody. The IgG portions included the hinge region, and the CH2 and CH3 domains. This chimeric receptor ("FGFR-IgG") bound both a human FGF and an anti-murine IgG antibody preparation. This chimeric fusion protein allowed the probe to bind novel ligands that exhibited critical minimal structural features of native FGF ligands that are required for receptor interaction and also to be detected using a secondary antibody. A

further advantage of this chimeric probe that included the FGFR ligand binding domain was that if allowed binding of structures as large as the native ligand or as small as the minimal critical structure needed for receptor binding.

Construction and Screening of a Phage Display Library

We used high complexity phage display libraries (about 1.8 X 10^8 variants/library) as the source of molecular diversity for ligand identification. These phage display libraries provided a rich source of molecular diversity which could be rapidly screened using high throughput screening *in vitro* assays.

In a typical phage display library, random peptides are displayed on the surface of bacteriophage M13 as N-terminal fusions expressed on major (e.g., pVIII) or minor (e.g., pIII) coat proteins. The N-terminal portion of the native pIII molecule binds the bacterial F pilus during infection and the C-terminal portion anchors the protein in the phage capsid. Individual bacteriophage particles displaying sequences having desirable binding characteristics can be affinity purified and cloned using standard laboratory techniques. Phage libraries are typically constructed to display random sequences of only 6 to 8 amino acids in length as fusion proteins. Longer random peptides displayed on phage libraries (*e.g.*, about 30 to 50 amino acid residues fused to the pIII protein), however, are advantageous because of the potential increased complexity due to a "sliding window" effect in any given peptide. For example, a 38-mer peptide contains 32 overlapping hexamers. Moreover, such peptides may be able to assume tertiary structures independent of the phage protein to which the peptides are fused. Such tertiary structures can represent discontinuous epitopes in complex proteins which may be important when screening for molecules capable of interacting with complex targets such as cell surface receptors. Moreover, libraries of random peptides make possible the formation of multiple contact sites that are capable of binding complex targets.

The libraries employed in the screening procedures of the present invention displayed fusion proteins comprising contiguous random amino acid sequences with the pIII minor coat protein of phage M13. The fusion proteins included insertions of random sequences of 8 to 43 amino acids to the N-terminus. Initially, four different M13 phage display libraries were screened for binding to the FGFR probe. These four libraries, designated RC8, RC13,

R38 and RC43, displayed pIII fusion proteins containing random sequences of eight, thirteen, 38 and 43 amino acids, respectively. Clones that bound to the FGFR probe were isolated and purified from the RC13, R38 and RC43 libraries using immunocapture methods that relied on binding to the FGFR probe as described in detail below. Briefly, the FGFR probe was incubated with the complete phage library to allow binding to the FGFR portion of the FGFR-IgG chimeric protein to random amino acid sequences presented on the phage. The phage bound with the FGFR probe were then isolated by binding anti-IgG antibodies immobilized on a solid support to the peptide:FGFR-IgG complex. The solid support with its bound peptide:FGFR-IgG:anti-IgG complex was isolated, non-binding phage were washed away and the bound phage were eluted from the solid support. The eluted phage were grown using standard bacteriophage procedures and the selection procedure was repeated. From these enriched phage, clones were isolated and assayed by ELISA for binding affinity to the FGFR probe. Then the phage DNA encoding the pIII fusion proteins of selected binding clones were sequenced to determine the amino acid sequences of the random peptide portions of the chimeric proteins.

An "evolved library" was constructed based on one selected initial clone. This phage display library was referred to as an "evolved library" because it represented clones that had been synthetically derived or evolved from the initial clone by a process of random substitution of nucleic acid bases in the nucleic acid encoding the peptide. Thus, the evolved library contained fusion peptides related to the peptide sequence of the initial clone.

Production and Screening of an Evolved Library

We selected an initial clone from the clones identified and isolated from the four initially-screened phage display libraries. The selected clone had been isolated from the RC13 library and its encoded peptide served as a lead peptide from which variants were derived and screened for binding to human FGFR. Molecular variants of the lead peptide were constructed in another library which was screened for clones that exhibited similar or enhanced binding activity. That is, we created a phage display library in which nearly all of the codon positions for the lead peptide were randomized at a predicted frequency of 50% to generate a library of variant fusion proteins. By screening the evolved library

of variants, we identified additional peptides that also bound the human FGF receptor probe. The DNA sequences of these binding variants were determined and the encoded peptide sequences were aligned to identify a consensus sequence of amino acids that possessed the desired receptor-binding characteristics. Herein, this process of creating libraries of variant peptides based on the nucleic acid sequence encoding a lead peptide is referred to as "molecular evolution."

Using this molecular evolution process, we screened greater than 100 million protein variants in phage to identify additional FGFR-binding peptides. Some of the clones isolated from the evolved library had increased affinity for the FGFR probe compared to that of the initial clone from whose sequence the evolved librAry was constructed. The nucleic acid coding sequences of the peptides in these clones were determined (SEQ ID NO:8 to SEQ ID NO:35). Alignment and comparison of the predicted amino acid sequences of these additional peptides and the lead peptide allowed identification of a consensus sequence of CXXLXXGAPFXXXXC (SEQ ID NO:1). This consensus sequence serves as a basis for synthesizing optimized receptor-binding peptides or peptide mimetics with similar or enhanced FGFR-binding properties. It will be appreciated by those skilled in the art that, when the initial clone contains a relatively long peptide, the optimized receptor-binding peptides or peptide mimetics may be and preferably are smaller than the size of the initial peptide 's amino acid sequence.

Identifying FGFR-Binding Peptides

We identified peptide mimetics of human FGF by screening four high complexity phage display libraries (each of about 1-2 X 10^8 diversity) to identify peptides that bind to a human FGF receptor probe. Phage having the desired binding activity were affinity purified from the vast excess of non-binding phage, and the DNA of the purified phage was isolated, amplified and sequenced to determine the amino acid sequence of the displayed peptide.

We screened phage display libraries displaying random-sequence peptides using an IgG-HFGF receptor fusion protein to identify and isolate initial and evolved human FGF peptide mimetics. Although the process employed below is generally applicable to the discovery of peptides that bind virtually any

receptor, particularly any type I cytokine or growth factor receptor, the structure of the peptides having binding activity is not predicted in advance of isolating and sequencing the binding clones 's DNA. That is, the clones are not constructed starting from a known ligand sequence.

By determining the DNA and predicted amino acid sequences of peptides in clones isolated from an evolved library that bound to the FGFR probe a consensus sequence of amino acids was identified as representative of the FGFR-binding variants isolated from the evolved library. This consensus amino acid sequence is $CX_1X_2LX_3X_4GAPFX_5X_6X_7X_8C$, (SEQ ID NO:1) in which X_1 is S or R, X_2 is any natural α-amino acid, X_3 is a neutral and hydrophobic α-amino acid, X_4 is any natural α-amino acid, X_5 is H or Q, X_6 is a branched-chain amino acid, X_7 is a neutral and hydrophobic or a basic α-amino acid, and X_8 is an acidic amino acid or a neutral and polar amino acid.

Although certain trends in FGFR-binding peptides may be discerned by examination of these sequences from which the consensus amino acid sequence was determined, particularly for preferred amino acids for some residues, these amino acids are not exclusive. That is, FGFR-binding peptides that retain the conserved consensus sequence, even if not represented by any of the individual sequences of the evolved binding clones that were isolated (SEQ ID NO: 37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, and SEQ ID NO:49), are within the scope of this invention.

Preferred methods and materials are described in the examples that follow. Standard methods that can be used by those skilled in the art to perform the genetic engineering and other procedures described herein are found in *Molecular Cloning: A Laboratory Manual* (Sambrook et al. eds. Cold Spring Harbor Lab Publ. 1989) and *Current Protocols in Molecular Biology* (Ausubel et al. eds., Greene Publishing Associates and Wiley-Interscience 1987) and other well known protocol references. Well known immunoassay methods including solid-phase immunoassays suitable for rapidly screening large numbers of peptides and other compounds are also known to those skilled in the art (*see* Harlow & Lane, *Antibodies, A Laboratory Manual*, 1988, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, particularly Chapt. 14, pp. 553-612).

The invention can be better understood by way of the following examples which are representative of the preferred embodiments. Equivalent methods and materials could be used to practice the invention and, therefore, the information included in the Examples should not to be construed as limiting the scope of the invention.

Example 1 describes construction of a plasmid cloning vector, pcDNA3-IgG1, that encoded a portion of the chimeric IgG-FGF protein used for probing phage display libraries. This plasmid vector encoded a portion of the murine IgG1 heavy chain and was designed to receive a polynucleotide cassette that encoded the ligand-binding domain of the huFGF.

Example 1

Expression Vector Encoding the C_{H2}, C_{H3} and Hinge Domains of the Murine IgG1 Heavy Chain

A plasmid vector, pcDNA3, contains neomycin and ampicillin drug resistance (selectable marker) genes, ColE1, f1 and SV40 origins of replication, and a multiple cloning site containing restriction endonuclease recognition sequences for Hind III, Kpn I, BstX I, EcoR I, EcoR V, Not I, Xho I, Xba I and Apa I, where a DNA fragment cloned into the multiple cloning site region can be expressed by utilizing a CMV promotor contained in the vector sequence (Invitrogen Corp., San Diego CA). The plasmid pcDNA3 was digested with Not I and Xho I restriction endonucleases and the digestion products separated electrophoretically on a 1% agarose gel using TBE buffer (89 mM Tris, pH 8.0, 89 mM boric acid, 2 mM EDTA (ethylene diamine tetraacetic acid)). The largest DNA fragment of the digest was gel-purified, ethanol precipitated, pelleted and dried briefly. The dried pellet of purified DNA fragment was resuspended in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA) and stored at -20°C. This linearized plasmid was used to receive a polynucleotide that encoded a portion of a murine immunoglobulin (Ig) heavy chain.

A polynucleotide encoding the constant region CH2, CH3 and hinge domains of the murine IgG1 heavy chain was amplified from genomic DNA using a PCR protocol employing primers having the following sequences.

First strand primer was (SEQ ID NO:2):

5'-AGCTTCGA**GC GGCCGC**CGTG CCCAGGGATT GTGGTTGTAA G-3'

The opposite strand primer was (SEQ ID NO:3):

5'-GATC**CTCGAG** TCATTTACCA GGAGAGTGGG AGAGGCT-3'

The underlined portion of SEQ ID NO:2 corresponds to a Not I restriction endonuclease cleavage site, and the bolded underlined portion of SEQ ID NO:3 corresponds to an Xho I restriction endonuclease cleavage site.

Mouse genomic DNA was prepared from a lysate of frozen NIH3T3 cells using standard laboratory procedures. Briefly, cells ($5x10^5$) were pelleted by centrifugation, washed with phosphate-buffered saline, resuspended in 100 µl of a hypotonic buffer (50 mM KCl, 10 mM Tris HCl (pH 8.4), I.5 mM $MgCl_2$) containing 0.5% (v/v) nonionic surfactant (TWEEN®20) and 10 µg of proteinase K. The mixture was incubated at 56°C for 45 minutes, heated to 95°C for 10 minutes and thereafter stored at 4°C.

The PCR reaction for amplifying the polynucleotide region encoding the CH2, CH3 and hinge domains of the murine IgG1 heavy chain was prepared by combining the following reagents in a sterile 0.6 ml microfuge tube in the following order: 10 µl of 10X PCR Buffer II (100 mM Tris HCl (pH 8.3), 500 mM KCl), 6 µl of 25 mM $MgCl_2$, 2 µl of a 10 mM solution of each dNTP, 2.5 µl of 10 nM murine IgG1 first strand primer (SEQ ID NO:2), 2.5 µl of 10 nM murine IgG1 opposite strand primer (SEQ ID NO:3), 0.5 µl (2.5 units) of a thermostable DNA polymerase (AMPLITAQ®, Perkin Elmer Corp., Foster City CA), 66.5 µl ultrapure water, and one wax bead. After incubating the reaction mixture at 70°C to melt the wax bead, 10 µl of the lysate containing the genomic DNA template was added to the tube. The reaction mixture was incubated 30 cycles as follows: 1 min at 94°C, 1 min at 55°C, and 1.5 min at 72°C (in a Perkin Elmer 480 Thermal Cycler). The completed reaction mixture was stored at 4°C until use.

Amplified DNA from the PCR reaction was gel purified by electrophoresis through a 1% agarose gel in TBE. The band corresponding to the amplified DNA was excised from the gel and eluted in 40 µl of water. The amplified IgG1 gene fragment of about 1 kb was then digested with Not I and Xho I restriction endonucleases, and the digestion products electrophoresed on a 1% agarose/TBE gel. The about 1 kb DNA fragment was again purified from the gel and eluted in 40 µl of water. The yield of the purified fragment was

determined by measuring the optical density of the solution at 260 nm (Beckman DU600 spectrophotometer).

The Xho I and Not I digested IgG1 PCR product was ligated into the Xho I and Not I digested pcDNA3 vector in a 20µl ligation reaction containing about 100 ng each of the pcDNA3 vector and IgG1 amplified DNA fragment, in 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 1 mM ATP, 25 µg/mL bovine serum albumin (BSA) and 1 unit of DNA ligase, incubated overnight at room temperature. A 1 µl aliquot of the ligation mix was used to transform competent *E. coli* cells (SURE® EPICUREAN COLI®, Stratagene, La Jolla, CA) according to standard laboratory procedures. Transformants were selected by growth on LB plates containing ampicillin. Plasmid DNA isolated from several independent clones was digested with Not I and Xho I and resolved on a 1% agarose/TBE analytical gel to check for the presence of the polynucleotide segment encoding the murine IgG1 constant and hinge regions. Plasmid DNA from clones containing the Not I/Xho I insert was prepared for nucleic acid sequencing.

Nucleic acid sequencing of the Not I/Xho I insert was performed using a dideoxy sequencing protocol. Sequencing reaction mixtures were run on a 4% acrylamide denaturing gels containing urea for 10 hours and the entire sequence of the fragment determined. After verifying that one of the clones, designated pcDNA3-IgG1 (SEQ ID NO:4), contained an insert having the proper sequence (i.e., murine IgG1 C_{H2}, C_{H3}, and hinge regions), a large-scale plasmid preparation was carried out.

The pcDNA3-IgG1 expression vector was used to create a new expression plasmid encoding a chimeric HFGF protein useful for probing phage display or other combinatorial libraries for ligands, as described in Example 2. It will be appreciated by those skilled in the art that the pcDNA3-IgG1 vector may be used as a recipient DNA for sequences encoding other peptides, thereby allowing one to easily create other chimeric proteins useful in similar types of probing assays. That is, the pcDNA3-IgG1 vector is a general purpose vector for producing chimeric proteins that include a portion of the murine

The following example describes the methods used to construct a plasmid expression vector that encoded a chimeric protein having the C_{H2}, C_{H3},

and hinge regions of murine IgG1 and the ligand-binding domain of the human HFGF.

Example 2

Expression Vector Encoding a Chimeric Receptor Incorporating the Human FGFR Ligand Binding Domain

A DNA fragment encoding the extracellular portion of the human FGFR was obtained by PCR amplification of cDNA which had been synthesized from human bone marrow total RNA. RNA was reverse transcribed in a reaction mixture that included: 1 µg RNA, 12.5 Mm of each dNTP, 50 mM Tris-HCl (pH 8.3), 40 mM KCl, 5 mM DTT (dithiolthreitol), 20 pmoles of random deoxyribonucleotide hexamers, and 100 units of reverse transcriptase (SUPERSCRIPT®, Life Technologies Gibco/BRL, Grand Island, NY). The mixture was incubated for 1 hour at 42°C, heat-treated at 95°C for 5 minutes and then stored at 4°C until use. PCR reactions were performed using the following primers.

The first strand primer was:

5'-GATCGGATCCGCCACCATGGGAGCTGGAAGTGCCTCC-3' (SEQ ID NO:5); and

the opposite strand primer was:

5'-ATTACATAGCGGCCGCGGTCATCACTGCCGGCCTCTC-3' (SEQ ID NO:6).

The first strand primer (SEQ ID NO:5) incorporated into the amplification product an ATG translation start codon (shown underlined above) and a Bam HI recognition sequence (shown bolded above) located immediately upstream of the start codon. The opposite strand primer (SEQ ID NO:6) introduced a Not I restriction endonuclease cleavage site (shown bolded above) at the downstream terminus of the amplified fragment.

PCR reactions were performed using conditions substantially as described in Example 1 but with the primers having the sequences of SEQ ID NO:5 and SEQ ID NO:6, and yielded an amplified FGFR DNA fragment having the sequence of SEQ ID NO:7. The amplified FGFR DNA fragment (*i.e.*, the PCR product) and the pcDNA3-IgG1 plasmid each were digested with Bam HI and Not I, and the large DNA fragments of each reaction were gel purified using methods as described in Example 1. The purified FGFR DNA fragment and

plasmid vector were then ligated, using ligation conditions substantially as described in Example 1, to yield the chimeric expression vector designated pcDNA3-IgG1-FGFR. This chimeric expression vector was transfected into competent *E. coli* cells using standard procedures, substantially as described in Example 1. RNA transcribed from the vector-borne CMV promoter was translated within the transfected cells to yield a fusion protein having domains corresponding to murine IgG1 and the extracellular domain (ECD) of the human FGFR. Vector construction was confirmed by diagnostic restriction digests and nucleic acid sequencing using standard methods. Large scale plasmid preparations were made from a transformed *E. coli* clone harboring the pcDNA3-IgG1-FGFR plasmid.

The following example describes the methods used to produce the chimeric FGFR-IgG protein encoded by the pcDNA3-IgG1-FGFR plasmid described above within eukaryotic cells. Although COS-7 host cells were used in these procedures, those skilled in the art will appreciate that a variety of other cultured cells may easily be substituted.

Example 3

Transfection of the pcDNA3-IgG1-FGFR Construct and Expression of the Chimeric FGFR-IgG Protein

COS-7 cells were propagated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 4500 mg/L D-glucose, 584 mg/ml L-glutamine, and 10% fetal bovine serum (FBS). In preparation for transfections, cells were seeded at about 2-4 x 10^5 cells per T-150 in about 20 ml volumes and grown in a humidified incubator at 37°C in a 5% CO_2 atmosphere until about 50% to 70% confluent. Plates were washed with serum-free media, and a mixture of 10 µg of pcDNA3-IgG1-FGFR plasmid DNA and 31 µl of standard transfection reagent comprising positively charged and neutral lipids (LIPOFECTAMINE®, GibcoBRL, Grand Island, NY) in DMEM was added to the cells, which were then returned to the incubator for 5 hrs. Transfection media subsequently was replaced with DMEM and 10% FBS. Stable transfectants were drug selected in the presence of G418 using standard laboratory procedures. After 48 to 72 hrs, cell free media was assayed for the presence of the FGFR-IgG protein using an ELISA protocol described in the following example. The results of those assays

showed that proteins in the cell free supernatants contained one moiety capable of binding to authentic FGFR and another moiety capable of binding to an anti-IgG antibody. That is, transfectants containing the pcDNA3-IgG1-FGFR plasmid expressed a chimeric protein that included functional IgG1 and FGFR regions.

Example 4 describes the ELISA methods used to confirm that the chimeric FGFR-IgG protein was secreted from cells transfected with the pcDNA3-IgG1-FGFR plasmid and describes the method used to demonstrate that the chimeric protein simultaneously could bind authentic FGFR and a labeled anti-IgG antibody.

Example 4

ELISA Protocol to Verify the Production and Integrity of the Chimeric FGFR-IgG Protein

The basic enzyme linked immunoabsorbent assay (ELISA) method for determining relative quantities of secreted proteins from transfectants was as follows. Wells of a standard 96-well plate (IMMULON®2, Dynatech) were coated with goat anti-murine IgG_{fc} that hAd been diluted 1/1,000 in PBS. After blocking the wells for nonspecific binding using a solution of 1% BSA and 0.05% TWEEN® 20 in PBS, serially diluted samples of the cell free supernatant from transfectants were added to the wells and allowed to contact the immobilized antibody. After removing unbound chimeric protein by washing, a horseradish peroxidase (HRP) labeled anti-murine IgG1 detection antibody was added and allowed to bind the IgG portion of the chimeric protein. The HRP labeled antibody, bound to the chimeric protein, was detected using a TMB peroxidase substrate (Kirkegard & Perry, Gaithersburg MD) using standard procedures. Activities were calculated by spectrophotometric measurements at 450 nm to 650 nm. That is, the concentration of the chimeric protein was calculated relative to a standard curve of known antibody. Specifically, absorbance of each dilution at 450 nm was measured and the absorbance at 650 nm was subtracted to eliminate absorbance from non-specific sources (e.g., cell debris and dust).

Results of these procedures indicated that protein concentrations ranged from 125 to 4,000 ng/ml depending on the confluency of the culture and the

frequency of supernatant harvesting. Having confirmed that transfected cells secreted a protein having a domain identifiable with anti-murine IgG antibodies, we proceeded to verify that the chimeric FGFR-IgG protein also could bind the FGF ligand.

In this ELISA, wells of a 96-well microtiter plate were coated with a 3.1 unit/ml solution of recombinant human HFGF (R & D Systems, Minneapolis MN) that had been diluted in PBS. The wells were washed three times with PBS containing 0.05% (v/v) TWEEN® 20 non-ionic detergent, and then blocked with 1% (w/v) BSA and 0.05% TWEEN® 20 in PBS (blocking buffer) to prevent non-specific binding. Excess blocking buffer was removed by washing once. Culture media was serially diluted in blocking buffer, and 50 µl from each dilution was added to the coated and blocked wells. Wells receiving undiluted media served as a control. A set of uncoated wells also received the diluted cell-free media. Plates were then incubated for 2 hours at room temperature, and washed three times as described above. After removing unbound chimeric receptor by washing, bound chimeric protein was assayed using 100 µl of the HRP-labeled anti-murine IgG antibody, substantially as described above. Color development was initiated by addition of 100 µl of the TMB peroxidase substrate, and the extent of the peroxidase reaction measured spectrophotometrically at 450 nm and 650 nm, as described above.

Results of these procedures indicated that the chimeric receptor protein bound to human FGF, and was detectable by using a labeled anti-murine IgG antibody. Control wells showed no color development, whereas wells in which an FGF/FGFR-IgG complex had been formed were distinctly blue to purple in color.

These results showed that the chimeric FGFR-IgG protein was suitable as a probe for identifying ligands specific for FGFR. That is, the chimeric FGFR-IgG exhibited specific binding to FGF, and also specifically bound an anti-murine IgG antibody.

The following example describes the methods used to purify the chimeric FGFR-IgG protein used to identify FGFR ligands.

Example 5

Purification of the Chimeric FGFR-IgG Protein

Sterile culture supernatants containing the chimeric FGFR-IgG protein were loaded onto an anti-murine IgG1 affinity column (SEPHAROSE® affinity column, Zymed Laboratories, San Francisco CA) equilibrated with PBS. After loading, the column was washed with phosphate-buffered saline solution (PBS) to remove non-specifically bound proteins. Proteins bound to the anti-IgG1 were removed from the column by eluting with a buffer containing 0.15 M NaCl and 0.1 M glycine (pH 2.4). Protein-containing fractions were pooled, concentrated and buffer exchanged into PBS using standard procedures (CENTRICON® 50 cartridge, Amicon, Beverly, MA). The relative protein concentration of the FGFR-IgG chimera was determined using an IgG sandwich ELISA and a known concentration of antibody, all according to standard laboratory procedures substantially as described in Example 4. The integrity of the chimeric protein was determined by PAGE on 4% to 20% Tris-glycine pre-cast acrylamide gels and silver staining (Bio-Rad, Hercules CA), and immunoblotting using HRP labeled goat anti-murine IgG1 to detect the FGFR-IgG protein, also according to standard laboratory procedures.

Results of these procedures indicated that the major protein species had a molecular weight of about 55 KDa. Contaminating bands and degradation products on the gel were minimal. The FGFR-IgG protein easily was recoverable in microgram amounts using the procedures described above.

Using this purified chimeric FGFR-IgG protein as a probe, we screening four phage display libraries (RC8, RC13, R38 and RC43) and isolated phage clones that bound the FGFR. For screening, the protein probe was immobilized to magnetic beads, although alternative solid supports could readily be used. The magnetic beads were employed because the high surface area of the matrix facilitated efficient contact between the immobilized probe and phage, and magnetic separation allowed efficient washing between processing steps.

Example 6 describes the methods used to isolate recombinant phage that expressed fusion proteins capable of binding the chimeric FGFR-IgG probe.

Example 6

Phage Library Screening

Four M13 phage display libraries which expressed random peptides as a pIII fusion protein were screened independently using a magnetic bead

screening protocol with the FGFR-IgG probe. These libraries, designated RC8, RC13, R38 and RC43, were constructed using previously described procedures (McConnell et al., *Molec. Diversity* 1:165-176, 1996). The RC8 and RC13 libraries displayed random amino acid sequences of 8 and 13 residues, respectively, flanked by cysteine residues, which allow formation of intramolcular disulfide loops. The R38 library expressed a fusion protein displaying 38 random amino acids surrounding an invariant alanine at position 22 in the peptide sequence. The RC43 library displayed 43-mer peptides comprising 40 random amino acids surrounding an invariant Gly-Cys-Gly sequence at residues 21, 22 and 23, which allows the potential for a displayed peptide to form intramolecular disulfide loops of varying sizes.

The phage libraries were screened for FGFR-binding clones using the above-described chimeric FGFR-IgG protein as a probe using immunocapture procedures in which the FGFR-IgG protein was bound to the phage in solution and then captured using anti-IgG immobilized to magnetic beads (DYNABEADS® M-45 rat anti-murine IgG1 beads, Dynal, Inc.). FGFR-IgG (about 500 ng/ml) was combined with 400 µl of magnetic beads, and the mixture incubated at 4°C overnight with gentle shaking. Beads were subsequently blocked for 1 hr at room temperature in standard Tris-buffered saline blocking medium (TBS SUPERBLOCK®, Pierce Chemical, Chicago IL). The coated beads were washed three times in TBST (Tris buffered saline containing 0.1% (v/v) TWEEN® 20) and then suspended in 1 ml blocking medium (TBS SUPERBLOCK®). Phage in 100 µl aliquots containing about 1×10^{10} plaque forming units (pfu) were incubated with the above-described FGFR-IgG coated beads (100 µl) in solution at room temperature for 2 hr, with gentle rotation. Beads with bound phage were collected by applying a magnetic field and, after washing the beads five times in TBST, phage were eluted with 300 µl of 50 mM glycine buffer (pH 2.2) for 10 min at room temperature. Eluted phage were immediately neutralized with 8 µl 2M Tris base that had not been pH-adjusted, mixed and stored at 4°C. Phage isolated by this immunocapture procedure were amplified by mixing equal volumes of eluted phage and an overnight culture of *E. coli* strain JS5, diluting the mixture in 20 ml 2xYT containing

tetracycline (12.0 µg/ml), and incubating overnight with shaking at 37°C. The resulting phage lysates were cleared of cell debris by centrifugation for 5 min in a microfuge, and 100 µl of the resulting supernatant lysate was used for a subsequent round of immunocapture as described above. All phage titering was carried out using JS5 bacteria plated on 2xYT top agar and agar plates.

Using these methods, fourteen positive binding clones were isolated from the RC13, R38 and RC43 libraries. The DNA sequences of these fourteen clones (SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, and SEQ ID NO:34) were determined for the pIII fusion proteins and the random peptide sequences contained therein were predicted based on the DNA sequences. The amino acid sequences of the random peptide portions encoded by these DNA sequences are shown in Table 1. Of these, clones 61, 64 and 66 were isolated from the R38 library; clones 73, 74, 83 and 94 were isolated from the RC43 library; and the other clones were isolated from the RC13 library.

Table 1

SEQ ID NO.	CLONE NO.	AMINO ACID SEQUENCE
9	61	SGTCSLDWVRGVRCYPGGQDASHPHGRPNAPRPSPTL
11	64	SGWEGTRLDPRGRYWGLSPTLDANIYSPRISNSARTQPN
13	66	REVQARDSRTFPQGGRLLTGGDATFHLETPPMIPTTLRP
15	73	SVGGVGDGEDLKHSDLAPVGGGCGTLFNCQSVASSSQPPPPLWN
17	74	RASRGWNDCVGVLPDKRPVPLGCGTSASRGATSKLTPTQVTRPS
19	83	SLRYTDTACRSGRNFTVCLDNSCGEHYTTSVSCSSVHDFTNAQQ

21	94	RPFGDGASKDGSVEEKEEAVTGCGTLFHCLPRHARFH RPSPTPA
23	13-1	CSALFVGAPFHVPDC
25	13-3	CLPALLGGPFAVPGC
27	13-5	CLPPFRSVSGGLLEC
29	13-6	CIRSSDPVGAVGLFC
31	13-9	CLFSAPLGDVMPLGC
33	13-10	CFGVSAKGAGVPPGC
35	13-12	CVPIGSGDSAFLSC

Although the amino acid sequences listed in Table 1 are not identical or similar to the amino acid sequence of human FGF, the phage clones bound specifically to the FGFR-IgG protein as shown in FIGS. 1A and 1B. A magnetic bead ELISA protocol was used to verify that the isolated phage clones bound to the chimeric FGFR-IgG probe and determine their relative binding affinities. The chimeric FGFR-IgG probe was bound to magnetic beads displaying rat anti-murine IgG1, substantially as described above. Samples (100 µl) of twelve 1:2 serial dilutions (PBS and 1% BSA) of the individual phage lysates were combined with the beads, and the phage allowed to bind the immobilized probe for 1 hr at room temperature. After removing unbound phage by washing four times with 200 µl aliquots of TBST (25 mM TrisHCl, pH 7.2, 0.15 M NaCl, 0.1% TWEEN® 20), an HRP-conjugated anti-M13 detection antibody (Pharmacia) diluted 1:5,000 in PBS was added. After incubating (1 hr at room temperature), excess detection antibody was removed by washing and 10 µl for each of the washed bead complexes was transferred to microtiter wells (FALCON® 3912, MICROTEST III, Beckton Dickinson, Oxnard, CA) that served as reaction vessels. Each well was developed using the TMB peroxidase substrate and the extent of the peroxidase reaction measured spectrophotometrically, as described in Example 4.

The results presented in FIG. 1A show the relative affinities of phage containing 43-mer peptides (clone 83, ×; clone 73, ●), 38-mer peptides (clone 64, ◆; clone 66, ▲) and 13-mer peptides (clone 13-1, ■; clone 13-9, *; clone

13-10, +; and clone 13-12, ▬). Similary, the results presented in FIG. 1B show the relative affinities of phage containing 43-mer peptides (clone 94, ▲; clone 74, ×), 38-mer peptides (clone 64, ◆; clone 61, ▬) and 13-mer peptides (clone 13-1, ■; clone 13-3, *; clone 13-6, ●; and clone 13-5, +). The inverse relationship between the optical density (O.D.) readout in the ELISA assay and the extent of phage dilution showed that progressively more dilute samples of the phage resulted in correspondingly less binding of the anti-M13 detection antibody. Control procedures indicated that the clones did not bind to the magnetic beads alone (results not shown). The results of FIGS. 1A and 1B show that clones 64 (38-mer) and 13-1 (13-mer flanked by C residues) had the highest relative affinities of the clones tested. Because of the relatively small size of the 13-1 clone peptide, this clone was selected as the initial isolate from which an evolved library was constructed.

To obtain phage clones having similar or higher affinity for the FGFR than the 13-1 clone, and to identify amino acids within the 13-1 fusion protein that contributed to FGFR binding, an evolved library was created using saturation oligonucleotide doping mutagenesis of the original 13-1 sequence and the evolved library was screened using the chimeric FGFR-IgG probe substantially asescribed above. The following example describes the methods used to create and screen an evolved phage display library of peptides that were related to the fusion protein of the 13-1 phage clone.

Example 7

Construction and Screening of an Evolved Library and Analysis of Additional Clones

An evolved library was constructed using procedures substantially as described previously (McConnell et al., 1996, *Molecular Diversity* 1:165). Briefly, we synthesized a collection of oligonucleotides such that codons corresponding to each amino acid position of the 13-mer internal to the flanking Cys residues of clone 13-1 were independently substituted by random amino acids at a predetermined frequency of approximately 50%.

FIG. 2 schematically shows the mutagenesis approach that was used to create this evolved peptide library from the clone 13-1 sequence. The upper line shows the amino acid sequence of the clone 13-1 peptide that was part of

the pIII fusion protein of the initial isolate. The DNA sequence thereunder shows the upper strand of a series of oligonucleotides that were synthesized in which the bases flanking the peptide were conserved in all the clones and are shown in capital letters, and the bases that were substituted are shown in small letters. For the first two positions of each codon of the 13-1 oligonucleotide, the evolved redundant oligonucleotides were synthesized using 73% of the nucleotide identical to the nucleotide present in that position of the 13-1 oligonucleotide sequence and 9% of each of the other three nucleotides. That is, redundant sequences for "g" residues were synthesized with a mixture of 73% G and 9% of each of A, T and C; for "a" residues were synthesized with 73% A and 9% of each of G, T and C; for "t" residues were synthesized with 73% T and 9% of each of G, A and C; for "c" residues were synthesized with 73% C and 9% of each of G, A and T; and for "s" residues were synthesized with 50% C and 50% C. This doping scheme produced nucleotide triplets encoding the original amino acid at each position of the 13-1 peptide approximately 50% of the time. The remaining 50% of the time, codons that did not encode the original amino acid were substituted by a random mixture of the other 19 amino acids.

Polynucleotides were synthesized according to the doping scheme diagramed in FIG. 2. Briefly, two collections of redundant oligonucleotides, represented by upper strand oligonucleotide shown in Fig. 2, and its complement (not shown) were synthesized so that oligonucleotides of the two groups could be annealed to each other using short complementary sequences present at their 3' ends (*e.g.*, the sequences shown as capital letters in FIG. 2 or their complement. The complementary strands of the annealed oligonucleotides were then synthesized by standard *in vitro* DNA extension methods to create a population of double-stranded DNA fragments. These DNA fragments were cloned into an appropriate M13 vector (e.g., MSM1 of McConnell et al., *supra*) to produce a phage display library that expressed pIII fusion proteins, where each fusion protein includes an N-terminal 13-mer peptide flanked by Cys residues, based on the 13-1 peptide sequence.

The resulting evolved library was screened using the immunocapture assay for clones capable of binding to the FGFR-IgG probe substantially as

described in Example 6. Several random phage clones also were isolated from the evolved library, to represent non-binding clones. As judged by the results of FGFR-specific magnetic bead ELISA testing (as described in Example 6), approximately 80% of clones selected from the enriched FGFR-binding group were positive for FGFR binding, whereas all of the randomly selected clones were negative for FGFR binding. This indicated that randomly selected clones in the evolved library were unlikely to bind the FGFR, whereas clones selected by the immunocapture procedure exhibited FGFR binding at a high frequency. Thus, the screening methods described herein are useful for identifying and isolating phage clones that contain ligands.

Seven FGFR-binding clones that were isolated from the evolved library had unique DNA sequences encoding the 13-mer peptide regions of the fusion proteins, as determined by standard dideoxy sequencing procedures. These unique DNA sequences (SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48) were used to predict the amino acid sequences of peptides shown in Table 2. The last entry in Table 2 shows the consensus amino acid sequence determined by comparison of the sequences of the 13-1 clone and the evolved clones (7, 11, 17, 40-43), where the X's represent positions tolerant of variable amino acids. Although the mutagenesis scheme used to produce these clones would produce codons unlike those encoding the original amino acid of the parent clone at a rate of 50% of the time, all of the binding clones sequenced included terminal C residues and the internal "GAPF" sequence, whereas none of the non-binder clones that were sequenced included these elements (data not shown). For some of the positions shown in the consensus sequence as "X" there is a preference for certain types of amino acids.

When the consensus amino acid sequence is shown as $CX_1X_2LX_3X_4GAPFX_5X_6X_7X_8C$, isolated evolved clones ' sequences show that X_1 is preferably S or R, X_3 is preferably a neutral and hydrophobic α-amino acid, X_5 is preferably H or Q, X_6 is preferably a branched-chain amino acid, X_7 is preferably a neutral and hydrophobic or a basic α-amino acid, and X_8 is an acidic amino acid or a neutral and polar amino acid. The consensus sequence includes two terminal cysteine residues capable of disulfide bridging to produce

a cyclic peptide, and four central invariant residues, although the other amino acid residues probably also contribute to binding of FGFR.

Table 2

CLONE	PEPTIDE	SEQ ID NO
Initial Clone 13-1	CSALFVGAPFHVPDC	23
Evolved Clone No. 7	CRVLIEGAPFHVPGC	37
Evolved Clone No. 11	CSVLLEGAPFQLPEC	39
Evolved Clone No. 17	CRALLRGAPFHLAEC	41
Evolved Clone No. 40	CRGLLAGAPFQVPDC	43
Evolved Clone No. 41	CRPLLLGAPFHVPEC	45
Evolved Clone No. 42	CSDLLVGAPFQLRGC	47
Evolved Clone No. 43	CSALLVGAPFQVAEC	49
CONSENSUS SEQUENCE	CXXLXXGAPFXXXXC	1

To better characterize the evolved clones having FGFR-binding specificity, we determined the relative affinities of the evolved clones using a phage ELISA procedure as shown by the results presented in Figure 3. Phage stocks of the FGFR-binding clones were titered in triplicate to accurately determine the concentration of pfu/ml for each lysate. Different isolates were then assayed by ELISA using normalized phage concentrations so that all clones were tested using equivalent numbers of phage. Optical densities (O.D.) in the ELISA corresponding to the equivalent of a 50% maximum response in the 13-1 clone were next determined for each of the different phage isolates. The optical density per phage at 50% 13-1 maximum response multiplied by $1x10^8$ gave a value of 1.0 for 13-1, and represented the standard for measuring relative binding affinity of the evolved clone isolates (clone nos. 7, 11, 17, 40-43). The relative affinities for each clone for ten serial dilutions ("PFU Dilution") are shown in FIG. 3.

As can be seen from FIG. 3, clones 7 (■), 11 (▲) and 17 (-●-) have affinities higher than the affinity of the parent 13-1 clone (-- --); clones 40 (▲) and 42 (--◆--) and 43 (-◆-) have affinities similar to that of the parent 13-1 clone;

and clone 41 (-- --) has an affinity less than that of the parent 13-1 clone. The different relative affinities probably result from amino acid differences between the different clones. Thus, many binding clones isolated from the evolved library bound to the ligand binding domain of the human FGF receptor with increased affinities relative to that the 13-1 parent clone. Because clone 17 showed the highest affinity in this assay, a peptide consisting of the clone 17 sequence (SEQ ID NO:41) was synthesized and assayed in a competitive binding assay.

The following example describes methods used to demonstrate that synthetic peptides having amino acid sequences derived from the sequences of the binding clones described above competed with authentic HFGF for binding to FGFR-IgG.

Example 8

Peptide Inhibition ELISA

This example shows that a synthetic peptide that includes the consensus sequence of SEQ ID NO:1 can compete with purified FGF for FGFR binding. The synthetic peptide tested was based on the peptide encoded by the DNA sequence of clone 17 (SEQ ID NO:40 and SEQ ID NO:41).

Synthetic peptide 17 having the sequence CRALLRGAPFHLAEC (SEQ ID NO:41) was synthesized in the cyclic disulfide-bonded form using standard procedures (Peninsula Laboratories, Belmont, CA). The 15-mer peptide structure was greater than 95% pure as judged by HPLC, and confirmed by mass spectrometry. Peptide 17 was tested in the FGFR-specific sandwich ELISA protocol described above for its ability to inhibit binding of the FGFR-IgG protein to bFGF-coated polystyrene plates (FALCON™). Also tested in the assay were recombinant human bFGF and an irrelevant 10-mer control peptide having the sequence CELPPGGGIC (SEQ ID NO:50).

The results presented in FIG. 4 show the degree of inhibition ("Percent Inhibition") for the protein and peptides at different concentrations ("Inhibitor Dilution" as defined in Table 3). The Clone 17 synthetic peptide (-▲-) competed with purified human bFGF for binding to the chimeric FGFR-IgG protein. Similarly, the recombinant human bFGF (■) was an effective competitor by binding to FGFR-IgG in solution, thus preventing binding to the

immobilized FGF. In contrast, the control peptide (--▲--) did not effectively compete with the FGFR probe for binding. The IC_{50} value of the Clone 17 peptide was about 3.9 μM in the assay. This proved that synthetic 15-mer peptide which included the consensus sequence identified based on all of the evolved FGFR-binding clones, bound the FGFR even when removed from the context of the M13 pIII fusion protein. Such synthetic peptides are FGF mimetics.

Table 3

Inhibitor Dilution	bFGF Concentration	Peptide 17 Concentration
10	3 nm	250 μm
9	1.5 nm	125 μm
8	750 pm	62.5 μm
7	375 pm	30 μm
6	188 pm	15 μm
5	94 pm	7.5 μm
4	47 pm	3.9 μm
3	24 pm	1.9 μm
2	12 pm	1 μm
1	6 pm	500 nm
0	3 pm	250 nm

Example 9

Synthetic FGF Peptide Mimetic Stimulates Proliferation in FGFR-Bearing Cells In Vitro

The biological activity of a representative synthetic peptide containing the 13-mer consensus sequence is tested for its ability to stimulate proliferation of human FGF-responsive cells. The Balb/C 3T3 cell line is used in this example because it is known to be simulated *in vitro* by aFGF and bFGF. Those having ordinary skill in the art will appreciate that other cell lines and primary cell lines (e.g., mesoderm-derived cells, Y1 adrenal cells, amniotic fluid-derived fibroblasts) are equally useful for detecting and quantitating the FGF peptide mimetic 's biological activity. The 3T3 cell line is widely known and commercially available from the ATCC.

3T3 cells are propagated in RPMI 1640 containing 10% BCS using standard tissue culture techniques. After washing twice with PBS to remove residual growth factors in BCS, 5,000 cells/well are placed in a 96-well microtiter plate in the presence of RPMI 1640 containing 0.5 units aFGF/ml (positive control), no additives (negative control), or the synthetic peptide based on clone 17 sequence as described in Example 8. The same synthetic peptide concentrations as used in FIG. 4 are tested in the tissue culture assay. Also tested as a negative control is an IL-6 peptide having the sequence GGAFCEAVGCGPDRNFYGG (SEQ ID NO:51) at the same concentrations as used for the clone 17 peptide. The cells are cultured for three days under standard conditions and cell proliferation in response to FGF or synthetic peptide is determined using a standard assay based on sodium 3'-(-1-(phenylaminocarbonyl)-3,4-tetrazolium)-bis (4-methoxy-6-notro) benzene sulfonic acid hydrate (XXT, Promega, Madison WI).

The results show that the FGF synthetic peptide derived from the clone 17 sequence exhibits FGF-like pharmacologic activity in a biological assay. The positive control, purified human aFGF, confirmed that the 3T3 cells are FGF responsive. The 3T3 cells proliferate with a half maximal response (ED_{50}) in the range of 0.05 to 0.1 unit/ml of aFGF. The synthetic clone 17 peptide is an FGFR agonist, producing proliferative responses in a dose-dependent manner in 3T3 cells over the concentration range tested. In contrast, an irrelevant IL-6

peptide that does not bind the FGFR does not stimulate 3T3 cell proliferation at any tested concentration. This shows that non-specific interactions in the assay do not contribute to the cell proliferative results seen with the synthetic FGF mimetic. Thus, synthetic peptides that include the consensus amino acid sequence (SEQ ID NO:12) exhibit a biological activity similar to native FGF.

While particular embodiments of the invention have been described in detail, it will be apparent to those skilled in the art that these embodiments are exemplary rather than limiting, and the true scope of the invention is defined by the claims that follow.

WHAT IS CLAIMED IS:

1. An isolated polypeptide capable of binding to a human Fibroblast Growth Factor receptor, having the formula $CX_1X_2LX_3X_4GAPFX_5X_6X_7X_8C$, wherein X_1 is S or R, X_2 is any natural α-amino acid, X_3 is a neutral and hydrophobic α-amino acid, X_4 is any natural α-amino acid, X_5 is H or Q, X_6 is a branched-chain amino acid, X_7 is a neutral and hydrophobic or a basic α-amino acid, and X_8 is an acidic amino acid or a neutral and polar amino acid.
2. The isolated polypeptide of Claim 1, wherein X_2 is a neutral and hydrophobic, neutral and polar, or acidic amino acid;
3. The isolated polypeptide of Claim 2, wherein X_2 is A, V, G, P or D.
4. The isolated polypeptide of Claim 1, wherein X_3 is a branched-chain amino acid or F.
5. The isolated polypeptide of Claim 4, wherein X_3 is L, I or F.
6. The isolated polypeptide of Claim 5, wherein X_3 is L.
7. The isolated polypeptide of Claim 1, wherein X_4 is a branched-chain amino acid, E, R or A.
8. The isolated polypeptide of Claim 7, wherein X_4 is V or E.
9. The isolated polypeptide of Claim 1, wherein X_6 is V or L.
10. The isolated polypeptide of Claim 1, wherein X_7 is P, A or R.
11. The isolated polypeptide of Claim 10, wherein X_7 is P or A.
12. The isolated polypeptide of Claim 11, wherein X_7 is P.
13. The isolated polypeptide of Claim 1, wherein X_8 is E, D or G.
14. The isolated polypeptide of Claim 13, wherein X_8 is E or D.
15. The isolated polypeptide of Claim 14, wherein X_8 is E.
16. The isolated polypeptide of Claim 1, having an amino acid sequence selected from the group consisting of SEQ ID NO:23, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47 and SEQ ID NO:49.

17. The isolated polypeptide of Claim 16, having the amino acid sequence of SEQ ID NO:41.

18. A method of activating a human Fibroblast Growth Factor (FGF) receptor, comprising the steps of:

contacting a cell having a human FGF receptor on it surface with at least one purified polypeptide according to Claim 1; and

allowing the polypeptide to bind to the human FGF receptor, thereby initiating activation of the human FGF receptor.

19. The method of Claim 17, wherein the polypeptide has the amino acid sequence of SEQ ID NO:23, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, or a combination of said polypeptides.

20. A method of activating a human Fibroblast Growth Factor (FGF) receptor, comprising the steps of:

providing a polypeptide according to Claim 1;

synthesizing and purifying a peptide memetic modeled on the polypeptide;

contacting a cell having a human FGF receptor on it surface with the purified peptide mimetic; and

allowing the peptide mimetic to bind to the human FGF receptor, thereby initiating activation of the human FGF receptor.

21. A method of inhibiting binding of a human Fibroblast Growth Factor (FGF) to a human Fibroblast Growth Factor receptor, comprising the steps of:

providing a polypeptide according to Claim 1, in sufficient quantity to compete with a human FGF for binding to a human FGF receptor; and

allowing the polypeptide to bind to the human FGF receptor, thereby inhibiting binding of a human FGF to the FGF receptor.

22. The method of Claim 21, wherein the polypeptide has the amino acid sequence of SEQ ID NO:41.

23. A method of inhibiting binding of a human Fibroblast Growth Factor (FGF) to a human FGF receptor, comprising the steps of:

providing a polypeptide according to Claim 1;

synthesizing and purifying a peptide memetic modeled on the polypeptide;

contacting a cell having a human FGF receptor on it surface with the purified peptide mimetic; and

allowing the peptide mimetic to bind to the human FGF receptor, thereby inhibiting binding of a human FGF to the human FGF receptor on the cell.

24. A method of discovering drugs that mimic human Fibroblast Growth Factor (FGF), comprising the steps of:

constructing a phage display library in which a fusion protein comprising a polypeptide consisting of a random sequence of amino acids and a phage protein;

screening the phage display library for at least one clone that binds to a human FGF receptor probe;

isolating an initial clone that binds to the human FGF receptor probe;

determining a nucleic acid sequence of the initial clone that codes for the polypeptide contained within the fusion protein;

constructing an evolved phage display library by mutagenesis *in vitro* of the nucleic acid sequence of the initial clone;

isolating additional clones that bind to a human FGF receptor probe from the evolved phage display library;

determining nucleic acid sequences from the additional clones isolated from the evolved phage library for individual sequences that code for a binding polypeptide contained within each additional clone 's fusion protein, thereby determining an amino acid sequence of each binding polypeptide;

comparing at least two amino acid sequences of two different binding polypeptides to identify a consensus amino acid sequence capable of binding to a human Fibroblast Growth Factor receptor; and

synthesizing a compound that includes or mimics the consensus amino acid sequence.

25. The method of Claim 24, wherein the human FGF receptor probe is a chimeric protein comprising a human FGF receptor amino acid sequence covalently linked to a mammalian immunoglobulin heavy chain amino acid sequence.

26. The method of Claim 24, wherein the synthesizing step synthesizes an organic compound.

27. The method of Claim 26, wherein the synthesizing step synthesizes a polypeptide having an amino acid sequence unlike a contiguous sequence of amino acids in human FGF.

28. The method of Claim 27, wherein the polypeptide is a cyclic polypeptide.

29. An isolated DNA sequence coding for a polypeptide capable of binding to a human FGF receptor, selected from the group consisting of: SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46 and SEQ ID NO:48.

30. An isolated polypeptide capable of binding to a human FGF receptor containing an amino acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47 and SEQ ID NO:49.

31. The isolated polypeptide of Claim 29, wherein the polypeptide is a cyclic polypeptide.

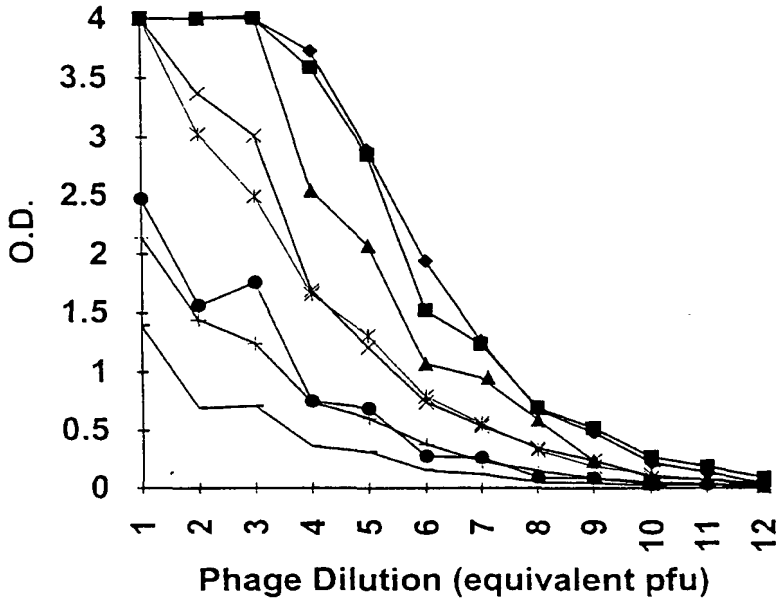
O.D.
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12
Phage Dilution (equivalent pfu)

FIG. 1A

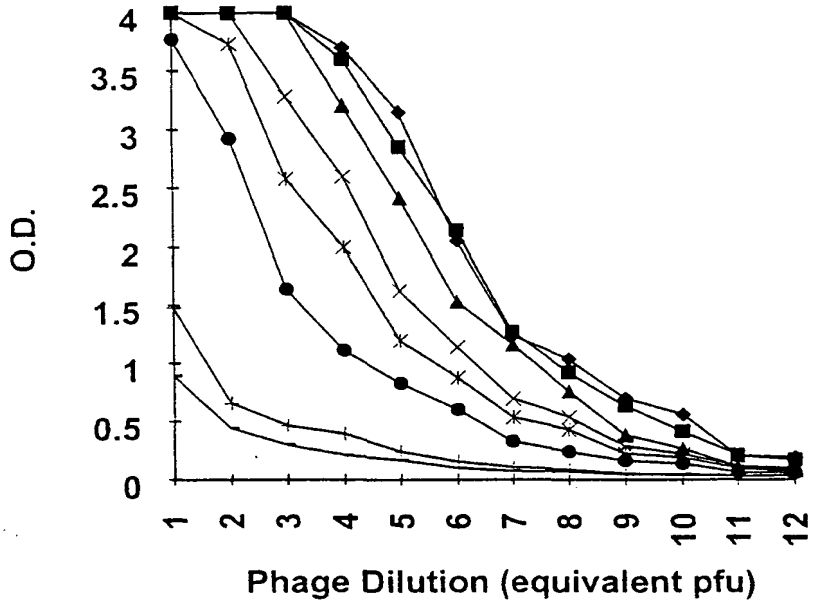
O.D.
4
3.5
3
2.5
2
1.5
1
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12
Phage Dilution (equivalent pfu)

FIG. 1B

FGFR Highest Affinity Phage Clone: Mutagenesis Scheme

C S A L F V G A P F H V P D C 13-1

G-TGT-GTC-TCG-AGC-tgs-ags-gcs-cts-tts-gts-ggs-gcs-ccs-tts-cas-gts-ccs-gas-tgs-TCT-AGA-GCG-CAC-GAC-TGT-CCC

AGA-TCT-CGC-GTG-CTG-ACA-GGG

FIG. 2

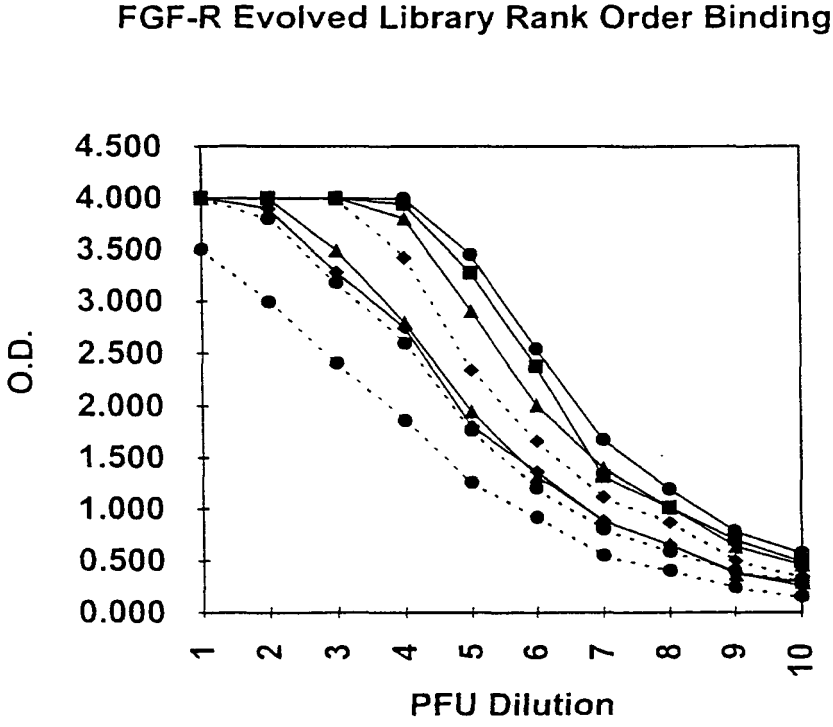
FGF-R Evolved Library Rank Order Binding
4.500
4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
O.D.
1
2
3
4
5
6
7
8
9
10
PFU Dilution

FIG. 3

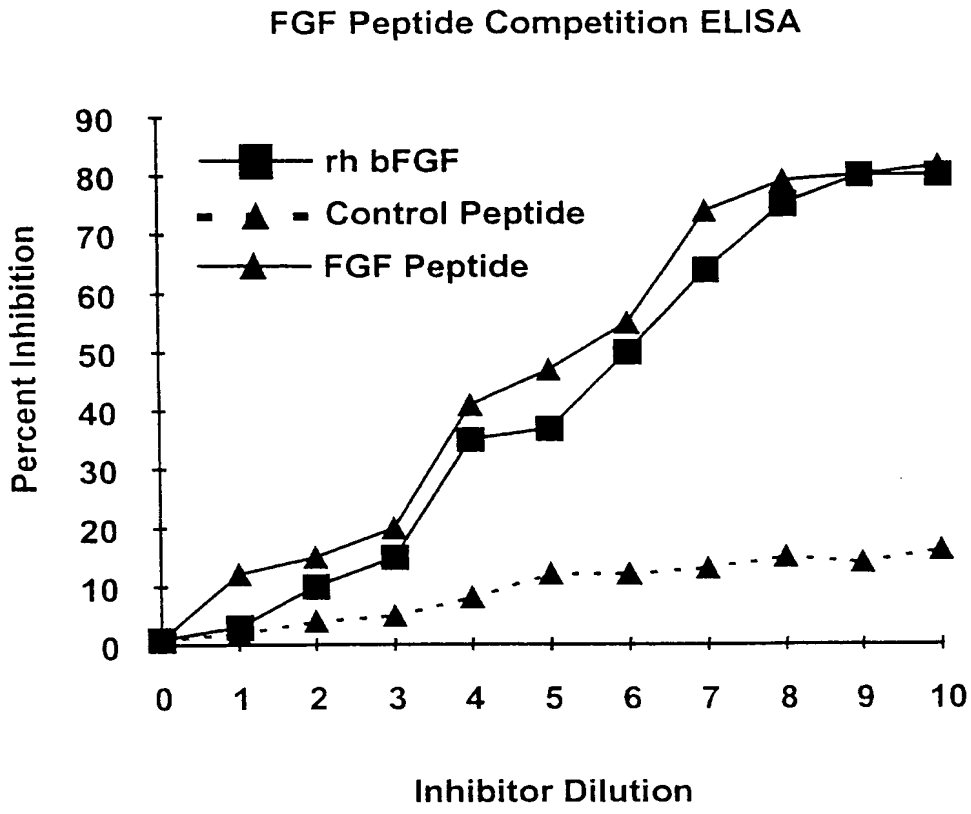
FGF Peptide Competition ELISA
Percent Inhibition
90
80
70
60
50
40
30
20
10
0
rh bFGF
Control Peptide
FGF Peptide
0 1 2 3 4 5 6 7 8 9 10
Inhibitor Dilution

FIG. 4

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: McCONNELL, Stephen
SPINELLA, Dominic
Chugai Pharmaceutical, Co., Ltd.

(ii) TITLE OF THE INVENTION: PEPTIDE LIGANDS FOR THE HUMAN FIBROBLAST GROWTH FACTOR (FGF) RECEPTOR

(iii) NUMBER OF SEQUENCES: 51

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Chugai Biopharmaceuticals, Inc.

(B) STREET: 6275 Nancy Ridge Drive

(C) CITY: San Diego

(D) STATE: CA

(E) COUNTRY: USA

(F) ZIP: 92121

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: Patent In

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: TBA

(B) FILING DATE: 28 May, 1999

(C) CLASSIFICATION: TBA

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/087,107

(B) FILING DATE: 28 May, 1999

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Hake, Richard A.

(B) REGISTRATION NUMBER: 37,343

(C) REFERENCE/DOCKET NUMBER: CH005-01.PCT

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 619-535-5944

(B) TELEFAX: 619-626-2574

(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Cys Xaa Xaa Leu Xaa Xaa Gly Ala Pro Phe Xaa Xaa Xaa Xaa Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AGCTTCGAGC GGCCGCCGTG CCCAGGGATT GTGGTTGTAA 40

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATCCTCGAG TCATTTACCA GGAGAGTGGG AGAGGCT 37

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6338 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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CGAGCAAAAT TTAAGCTACA ACAAGGCAAG GCTTGACCGA CAATTGCATG AAGAATCTGC 180

TTAGGGTTAG GCGTTTTGCG CTGCTTCGCG ATGTACGGGC CAGATATACG CGTTGACATT 240

GATTATTGAC TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA 300

TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC 360

CCCGCCCATT GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC 420

ATTGACGTCA ATGGGTGGAC TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT 480

ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT 540

ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA 600

TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG 660

ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC 720

AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG 780

GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTCT CTGGCTAACT AGAGAACCCA 840

CTGCTTACTG GCTTATCGAA ATTAATACGA CTCACTATAG GGAGACCCAA GCTGGCTAGC 900

GTTTAAACTT AAGCTTGGTA CCGAGCTCGG ATCCACTAGT CCAGTGTGGT GGAATTCTGC 960

AGATATCCAG CACAGTGGCG GCCGCCGTGC CCAGGGATTG TGGTTGTAAG CCTTGCATAT 1020

GTACAGGTAA GTCAGTGGCC TTCACCTGAC CCAGATGCAA CAAGTGGCAA TGGTTGGAGG 1080

GTGGCCAGGT ATTGACCTAT TTCCACCTTT CTTCTTCATC CTTAGTCCCA GAAGTATCAT 1140

CTGTCTTCAT CTTCCCCCCA AAGCCCAAGG ATGTGCTCAC CATTACTCTG ACTCCTAAGG 1200

TCACGTGTGT TGTGGTAGAC ATCAGCAAGG ATGATCCCGA GGTCCAGTTC AGCTGGTTTG 1260

TAGATGATGT GGAGGTGCAC ACAGCTCAGA CGCAACCCCG GGAGGAGCAG TTCAACAGCA 1320

CTTTCCGCTC AGTCAGTGAA CTTCCCATCA TGCACCAGGA CTGGCTCAAT GGCAAGGAGT 1380

TCAAATGCAG GGTCAACAGT GCAGCTTTCC CTGCCCCCAT CGAGAAAACC ATCTCCAAAA 1440

CCAAAGGTGA GAGCTGCAGT GTGTGACATA GAAGCTGCAA TAGTCAGTCC ATAGACAGAG 1500

CTTGGCATAA CAGACCCCTG CCCTGTTCGT GACCTCTGTG CTGACCAATC TCTTTACCCA 1560

CCCACAGGCA GACCGAAGGC TCCACAGGTG TACACCATTC CACCTCCCAA GGAGCAGATG 1620

GCCAAGGATA AAGTCAGTCT GACCGCCATG ATAACAGACT TCTTCCCTGA AGACATTACT 1680

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TGATCAGCCT CGACTGTGCC TTCTAGTTGC CAGCCATCTG TTGTTTGCCC CTCCCCCGTG 1980

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CAAAGCATGC ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA 2760

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TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TCCCGGGAGC TTGTATATCC 3000

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CTTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC TGCAGGACGA GGCAGCGCGG 3240

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GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC AGGATCTCCT GTCATCTCAC 3360

CTTGCTCCTG CCGAGAAAGT ATCCATCATG GCTGATGCAA TGCGGCGGCT GCATACGCTT 3420

GATCCGGCTA CCTGCCCATT CGACCACCAA GCGAAACATC GCATCGAGCG AGCACGTACT 3480

CGGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG AAGAGCATCA GGGGCTCGCG 3540

CCAGCCGAAC TGTTCGCCAG GCTCAAGGCG CGCATGCCCG ACGGCGAGGA TCTCGTCGTG 3600

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GCCGCTCCCG ATTCGCAGCG CATCGCCTTC TATCGCCTTC TTGACGAGTT CTTCTGAGCG 3840

GGACTCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA CCTGCCATCA CGAGATTTCG 3900

ATTCCACCGC CGCCTTCTAT GAAAGGTTGG GCTTCGGAAT CGTTTTCCGG GACGCCGGCT 3960

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GAAATTGTTA TCCGCTCACA ATTCCACACA ACATACGAGC CGGAAGCATA AAGTGTAAAG 4260

CCTGGGGTGC CTAATGAGTG AGCTAACTCA CATTAATTGC GTTGCGCTCA CTGCCCGCTT 4320

TCCAGTCGGG AAACCTGTCG TGCCAGCTGC ATTAATGAAT CGGCCAACGC GCGGGGAGAG 4380

GCGGTTTGCG TATTGGGCGC TCTTCCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTCG 4440

TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA TCCACAGAAT 4500

CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC AGGAACCGTA 4560

AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA 4620

ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC 4680

CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT 4740

CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCAATG CTCACGCTGT AGGTATCTCA 4800

GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG 4860

ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT 4920

CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA GGCGGTGCTA 4980

CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT 5040

GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC 5100

AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA 5160

AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG TGGAACGAAA 5220

ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG GATCTTCACC TAGATCCTTT 5280

TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA 5340

GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA 5400

TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC 5460

CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA 5520

ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC GCCTCCATCC 5580

AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA 5640

ACGTTGTTGC CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCTTCAT 5700

TCAGCTCCGG TTCCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG 5760

CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC 5820

TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTTT 5880

CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT 5940

GCTCTTGCCC GGCGTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC 6000

TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT 6060

CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA 6120

GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA ATAAGGGCGA 6180

CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG 6240

GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG 6300

TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTC 6338

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCGGATCC GCCACCATGG GAGCTGGAAG TGCCTCC 37

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTACATAGC GGCCGCGGTC ATCACTGCCG GCCTCTC 37

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 837 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGTGGAGCT GGAAGTGCCT CCTCTTCTGG GCTGTGCTGG TCACAGCCAC ACTCTGCACC 60

GCTAGGCCGT CCCCGACCTT GCCTGAACAA GATGCTCTCC CCTCCTCGGA GGATGATGAT 120

GATGATGATG ACTCCTCTTC AGAGGAGAAA GAAACAGATA ACACCAAACC AAACCCCGTA 180

GCTCCATATT GGACATCCCC AGAAAAGATG GAAAAGAAAT TGCATGCAGT GCCGGCTGCC 240

AAGACAGTGA AGTTCAAATG CCCTTCCAGT GGGACCCCAA ACCCCACACT GCGCTGGTTG 300

AAAAATGGCA AAGAATTCAA ACCTGACCAC AGAATTGGAG GCTACAAGGT CCGTTATGCC 360

ACCTGGAGCA TCATAATGGA CTCTGTGGTG CCCTCTGACA AGGGCAACTA CACCTGCATT 420

GTGGAGAATG AGTACGGCAG CATCAACCAC ACATACCAGC TGGATGTCGT GGAGCGGTCC 480

CCTCACCGGC CCATCCTGCA AGCAGGGTTG CCCGCCAACA AAACAGTGGC CCTGGGTAGC 540

AACGTGGAGT TCATGTGTAA GGTGTACAGT GACCCGCAGC CGCACATCCA GTGGCTAAAG 600

CACATCGAGG TGAATGGGAG CAAGATTGGC CCAGACAACC TGCCTTATGT CCAGATCTTG 660

AAGACTGCTG GAGTTAATAC CACCGACAAA GAGATGGAGG TGCTTCACTT AAGAAATGTC 720

TCCTTTGAGG ACGCAGGGGA GTATACGTGC TTGGCGGGTA ACTCTATCGG ACTCTCCCAT 780

CACTCTGCAT GGTTGACCGT TCTGGAAGCC CTGGAAGAGA GGCCGGCAGT GATGACC 837

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 111 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCTGGTACCT GCTCTCTGGA CTGGGTTCGT GGTGTTCGTT GCTACCCGGG TGGTCAGGAC 60

GCTTCTCACC CGCACGGTCG TCCGAACGCT CCGCGTCCGT CTCCGACCCT G 111

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Gly Thr Cys Ser Leu Asp Trp Val Arg Gly Val Arg Cys Tyr Pro
1 5 10 15

Gly Gly Gln Asp Ala Ser His Pro His Gly Arg Pro Asn Ala Pro Arg
20 25 30

Pro Ser Pro Thr Leu
35

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCTGGTTGGG AAGGTACCCG TCTGGACCCG CGTGGTCGTT ACTGGGGTCT GTCTCCGACC 60

CTGGACGCTA ACATCTACTC TCCGCGTATC TCTAACTCTG CTCGTACCCA GCCGAAC 117

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ser Gly Trp Glu Gly Thr Arg Leu Asp Pro Arg Gly Arg Tyr Trp Gly
1 5 10 15

Leu Ser Pro Thr Leu Asp Ala Asn Ile Tyr Ser Pro Arg Ile Ser Asn
20 25 30

Ser Ala Arg Thr Gln Pro Asn
35

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 117 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGTGAAGTTC AGGCTCGTGA CTCTCGTACC TTCCCGCAGG GTGGTCGTCT GCTGACCGGT 60

GGTGACGCTA CCTTCCACCT GGAAACCCCG CCGATGATCC CGACCACCCT GCGTCCG 117

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Arg Glu Val Gln Ala Arg Asp Ser Arg Thr Phe Pro Gln Gly Gly Arg

1 5 10 15

Leu Leu Thr Gly Gly Asp Ala Thr Phe His Leu Glu Thr Pro Pro Met

20 25 30

Ile Pro Thr Thr Leu Arg Pro

35

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 132 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCTGTTGGTG GTGTTGGTGA CGGTGAAGAC CTGAAACACT CTGACCTGGC TCCGGTTGGT 60

GGTGGTTGCG GTACCCTGTT CAACTGCCAG TCTGTTGCTT CTTCTTCTCA GCCGCCGCCG 120

CCGCTGTGGA AC 132

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ser Val Gly Gly Val Gly Asp Gly Glu Asp Leu Lys His Ser Asp Leu
1 5 10 15

Ala Pro Val Gly Gly Gly Cys Gly Thr Leu Phe Asn Cys Gln Ser Val
20 25 30

Ala Ser Ser Ser Gln Pro Pro Pro Pro Leu Trp Asn
35 40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 132 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGTGCTTCTC GTGGTTGGAA CGACTGCGTT GGTGTTCTGC CGGACAAACG TCCGGTTCCG 60

CTGGGTTGCG GTACCTCTGC TTCTCGTGGT GCTACCTCTA AACTGACCCC GACCCAGGTT 120

ACCCGTCCGT CT 132

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Arg Ala Ser Arg Gly Trp Asn Asp Cys Val Gly Val Leu Pro Asp Lys
1 5 10 15

Arg Pro Val Pro Leu Gly Cys Gly Thr Ser Ala Ser Arg Gly Ala Thr
20 25 30

Ser Lys Leu Thr Pro Thr Gln Val Thr Arg Pro Ser
35 40

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 132 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TCTCTGCGTT ACACCGACAC CGCTTGCCGT TCTGGTCGTA ACTTCACCGT TTGCCTGGAC 60

AACTCTTGCG GTGAACACTA CACCACCTCT GTTTCTTGCT CTTCTGTTCA CGACTTCACC 120

AACGCTCAGC AG 132

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ser Leu Arg Tyr Thr Asp Thr Ala Cys Arg Ser Gly Arg Asn Phe Thr

1 5 10 15

Val Cys Leu Asp Asn Ser Cys Gly Glu His Tyr Thr Thr Ser Val Ser

20 25 30

Cys Ser Ser Val His Asp Phe Thr Asn Ala Gln Gln

35 40

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 132 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGTCCGTTCG GTGACGGTGC TTCTAAAGAC GGTTCTGTTG AAGAAAAAGA AGAAGCTGTT 60

ACCGGTTGCG GTACCCTGTT CCACTGCCTG CCGCGTCACG CTCGTTTCCA CCGTCCGTCT 120

CCGACCCCGG CT 132

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Arg Pro Phe Gly Asp Gly Ala Ser Lys Asp Gly Ser Val Glu Glu Lys
1 5 10 15

Glu Glu Ala Val Thr Gly Cys Gly Thr Leu Phe His Cys Leu Pro Arg
20 25 30

His Ala Arg Phe His Arg Pro Ser Pro Thr Pro Ala
35 40

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGCTCTGCTC TGTTCGTTGG TGCTCCGTTC CACGTTCCGG ACTGC 45

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Cys Ser Ala Leu Phe Val Gly Ala Pro Phe His Val Pro Asp Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TGCCTGCCGG CTCTGCTGGG TGGTCCGTTC GCTGTTCCGG GTTGC 45

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Cys Leu Pro Ala Leu Leu Gly Gly Pro Phe Ala Val Pro Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TGCCTGCCGC CGTTCCGTTC TGTTTCTGGT GGTCTGCTGG AATGC 45

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Cys Leu Pro Pro Phe Arg Ser Val Ser Gly Gly Leu Leu Glu Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TGCATCCGTT CTTCTGACCC GGTTGGTGCT GTTGGTCTGT TCTGC 45.

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Cys Ile Arg Ser Ser Asp Pro Val Gly Ala Val Gly Leu Phe Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TGCCTGTTCT CTGCTCCGCT GGGTGACGTT ATGCCGCTGG GTTGC 45

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Leu Phe Ser Ala Pro Leu Gly Asp Val Met Pro Leu Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TGCTTCGGTG TTTCTGCTAA AGGTGCTGGT GTTCCGCCGG GTTGC 45

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Cys Phe Gly Val Ser Ala Lys Gly Ala Gly Val Pro Pro Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TGCGTTCCGA TCGGTTCTGG TGACTCTGCT TTCCTGTCTT GC 42

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Cys Val Pro Ile Gly Ser Gly Asp Ser Ala Phe Leu Ser Cys

1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TGCCGTGTTC TGATCGAAGG TGCTCCGTTC CACGTTCCGG GTTGC 45

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Cys Arg Val Leu Ile Glu Gly Ala Pro Phe His Val Pro Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TGCTCTGTTC TGCTGGAAGG TGCTCCGTTC CAGCTGCCGG AATGC 45

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Cys Ser Val Leu Leu Glu Gly Ala Pro Phe Gln Leu Pro Glu Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGCCGTGCTC TGCTGCGTGG TGCTCCGTTC CACCTGGCTG AATGC 45

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Cys Arg Ala Leu Leu Arg Gly Ala Pro Phe His Leu Ala Glu Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TGCCGTGGTC TGCTGGCTGG TGCTCCGTTC CAGGTTCCGG ACTGC 45

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Cys Arg Gly Leu Leu Ala Gly Ala Pro Phe Gln Val Pro Asp Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

TGCCGTCCGC TGCTGCTGGG TGCTCCGTTC CACGTTCCGG AATGC 45

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Cys Arg Pro Leu Leu Leu Gly Ala Pro Phe His Val Pro Glu Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TGCTCTGACC TGCTGGTTGG TGCTCCGTTC CAGCTGCGTG GTTGC 45

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Cys Ser Asp Leu Leu Val Gly Ala Pro Phe Gln Leu Arg Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TGCTCTGCTC TGCTGGTTGG TGCTCCGTTC CAGGTTGCTG AATGC 45

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Cys Ser Ala Leu Leu Val Gly Ala Pro Phe Gln Val Ala Glu Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Cys Glu Leu Pro Pro Gly Gly Gly Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Gly Ala Phe Cys Glu Ala Val Gly Cys Gly Pro Asp Arg Asn Phe
1 5 10 15
Tyr Gly Gly

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11844

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/53; A61K 38/00

US CL : 530/300, 326; 930/120; 435/7.1, 336

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 326; 930/120; 435/7.1, 336

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, WPIDS, WEST 1.2, SCISEARCH, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T,E	Database MEDLINE on Dialog, US National Library of Medicine, (Bethesda, MD, USA), No. 99349454, MCCONNELL. S. J. et al. Isolation of Fibroblast Growth factor Receptor Binding Sequence Using Evolved Phage Display Libraries. Comb. Chem. High Throughput Screen. June 1999, Vol. 2. No. 3, pages 155-63 (Abstract only).	1 and 18-20
A	YAYON. et al. Isolation of Peptides that Inhibit Binding of basic Fibroblast Growth Factor to its Receptor from a Random Phage-epitope Library. Proc. Natl. Acad. Sci. USA. November 1993, Vol. 90, pages 10643-10647.	1 and 18-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
08 SEPTEMBER 1999	04 NOV 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer P. PONNALURI Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11844

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1 and 18-20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.